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#19



Patent Docket P1780P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Kathy L. Miller et al. Serial No.: 09/813,341 Filed: March 20, 2001 Title: Multivalent Antibodies and Uses Therefor	Group Art Unit: 1642 Examiner: Misook Yu Confirmation No: 1230 Customer No: 09157 RECEIVED OCT 02 2003 TECH CENTER 1600/2003
	EXPRESS MAIL LABEL NO.: EV 351 926 794 US DATE OF DEPOSIT: <u>SEPTEMBER 26, 2003</u>

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

DECLARATION UNDER 37 CFR §1.131

Sir:

We, Kathy L. Miller and Leonard G. Presta, do hereby declare and say as follows:

1. We are inventors of the subject matter of the above-identified patent application. All work described hereinafter was performed by us or on our behalf in the United States of America.
2. Prior to July 14, 1999, we conceived of and reduced to practice an isolated antibody comprising an Fc region and three or more antigen binding sites amino-terminal to the Fc region.
3. Evidence of the reduction to practice of the claimed invention is set forth in the exhibits attached to this declaration which represent excerpts from our laboratory notebooks with dates obscured.

4. Exhibit A provides four laboratory notebook entries, pages 9, 10, 11 and 43, by Kathy L. Miller.

JP
9/24/03
Fabs in tandem
A diagram of a vector for tetravalent antibody 4H6oct is shown on page 9, in which vector the two ~~arms~~ of each polypeptide (boxes 1 and 2) are cloned amino-terminal to an Fc region. Expression of

this vector and purification of the resultant protein (page 10) yielded the 4H6oct antibody (page 11).

KM
09-26-03
Also shown on notebook page 11 is a gel isolation of tetravalent antibody OctHer2. Expression of a similar vector, but having 4H6 (arm 1) and 16E2 (arm 2) cloned amino-terminal to an Fc region yielded the tetravalent 4H6/16E2oct antibody shown on notebook page 43. The terms 4H6 and 16E2 refer to the DR4 and DR5 antigen binding regions, respectively, as shown on notebook page 43. The experimental work in Exhibit A was completed prior to July 14, 1999.

We declare further that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 09-26-03

Kathy L. Miller
Kathy L. Miller

Date: Sept. 26, 2003

Leonard G. Presta
Leonard G. Presta

Exhibit A

Project No. _____
Book No. **31884**

9

TITLE _____

From Page No. **6**

SET-UP PCR RXN TO SEQUENCE 4H60CT CORRECTED BY MUT. VIA ABT

(ALSO SEQU. 5EF26)
(BOOK 31885 p. 7)

PCR RXN		PGEM CNTL
BIG DYE RXN	8 μ l	8 μ l
TEMPLATE (500 ng)		2 μ l
PRIMER (NEAT)	1 μ l	4 μ l
DMSO	1 μ l	1 μ l
H ₂ O		5 μ l
	20 μ l	20 μ l

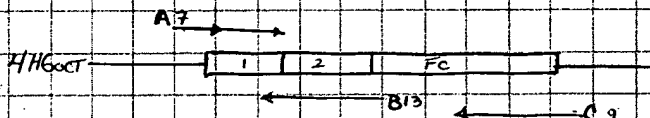
4H60CT-MUT

9-9#7 PRIMERS: A = KMSEQ.7
9-16#1 B = spigg 13L
9-16#3 C = spigg 9A

4H60CT-MUT	ng/ μ l	DILUTION	\approx 500ng	H ₂ O
9-9#7	2317	1:3	1 μ l	9 μ l
9-16#3	903	1:2	1 μ l	9 μ l
9-16#1	2397	1:3	1 μ l	9 μ l

LANE#
ON GEL

21 9-7A
22 9-7B
23 9-7C
24 16-3A
25 16-3B
26 16-3C
27 16-1A
28 16-1B
29 16-1C



PROCESS SAMPLES

- 1) 250 μ l 82G-50 SEPHAROSE/MINICOLUMN (in MEME TUBE)
- 2) SPIN 2' 3rpm. PLACE COLUMN IN FRESH MEME TUBE
- 3) ADD SAMPLE W/O DISTURBING BED. SPIN 2.5' 3rpm
- 4) DRY BY VAPORIZATION \approx 30'
- 5) RESUSPEND in 6 μ l LOADING DYE
- 6) INCUBATE 95°C 2'; ON ICE
- 7) LOAD 2 μ l

PAUSE; LOAD ODDS#; RUN INTO GEL; PAUSE; CLEAN WELLS, LOAD EVEN#S, RUN TO TERMINATE

WHEN PRESS RUN, ASKS TO "SAVE AS: GELFILE-DATE"

To Page No. _____

Witnessed & Understood by me, _____

Date _____

Invented by _____

Date _____

Recorded by _____

From Page No. 8CONCENTRATE + PURIFY OCTOPUS PROTEIN CONSTRUCTS

- 1) Collect SUPS FROM TRANSFECTED CELLS INTO 250ml CONICAL FLASK, CENTRIFUGE 2500rpm 5' 4°C
- 2) TRANSFER SUPS TO T75 FLASK PER 50ml ADD: 500 μ l 0.1M PMSF (0.001M)
50 μ l 50mM AC279 1.9mg/ml APROTININ
- 9 x 25 = 225ml
4.5 x 500 = 2.25ml PMSF
x 50 = 225 μ l APROTININ
- 3) CONCENTRATE USING SPIN-FILTER CONC. WITH YM100 DIAFILTERS; CONC. TO 10-15% STARTING VOL
- 4) WASH PROTEIN A COLUMN WITH 10ml 1X BINDING BUFFER (0.5ml/BED VOL)
- 5) ADD SAMPLE TO COLUMN
- 6) WASH COLUMN w/ 10ml 1X BINDING BUFFER
- 7) ELUTE w/ 2.5ml ELUTION BUFFER INTO TUBE CONTAINING 500 μ l COLD 1M TRIS, pH 8.0, TO NEUTRALIZE THE ACID ELUTION
- 8) CONCENTRATE TO \approx 1ml in PBS USING CENTRIFUGER - 30 (CONC + BUFF EXCHANGE w/ 2 x 15ml PBS)
- 9) FILTER w/ MILLER GV0.22 μ m FILTER

	OD 280	1:10 DILUTION	EXT. COEFF 1.38 (OTHER 2)
OCTHER 2	0.502	3.6 mg/ml	(OD 280) (10) = mg/ml
4H6 OCT 9-9-7	0.005		1.38
* \rightarrow 9-16-1	0.098	0.7 mg/ml	
9-16-3	0.012		

To Page No. Witnessed & Understood by me, Date Invented by Recorded by Date

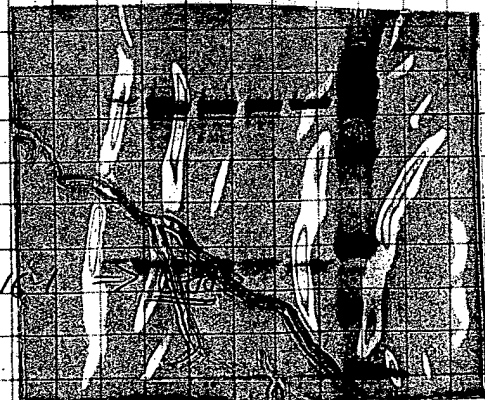
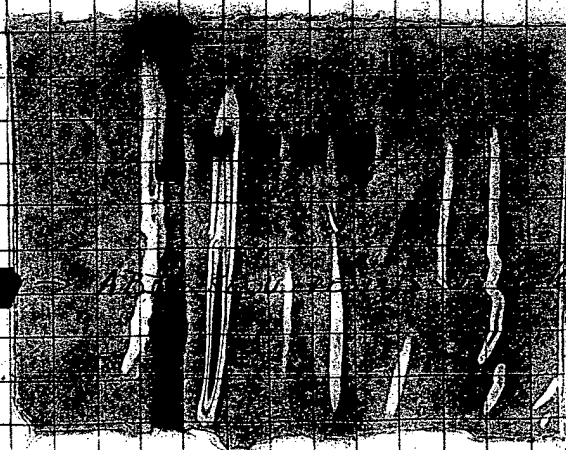
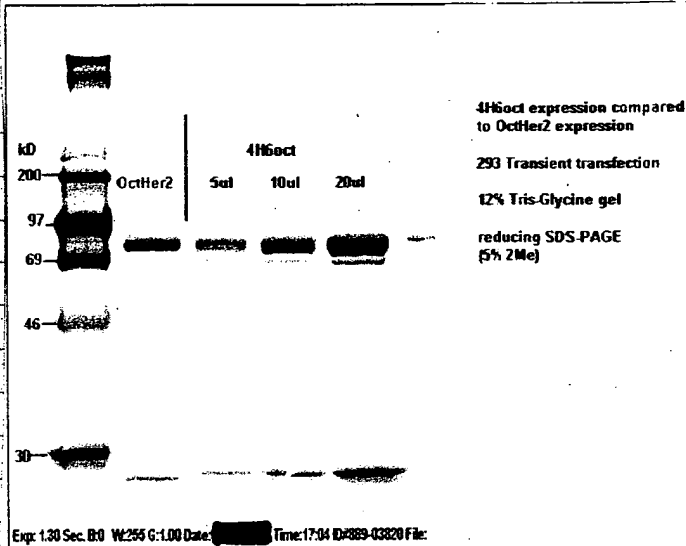
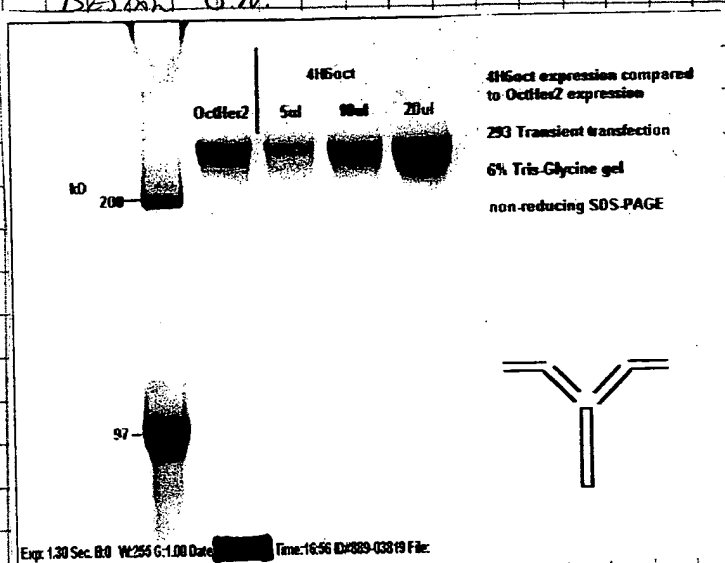
TITLE _____

From Page No. _____

Coomassie Stain of SDS-PAGE DECIDED TO LOOK AT 4H6oct 9-16-1 → HIGHEST CONC.

14% TRIS-GLYCINE @ 2ME REDUCING
6% TRIS-GLYCINE @ 2ME NONREDUCING

BOIL SAMPLES 5'; QUICK-SPIN; RUN 40 MINUTES ON ICE
STAIN 2 NLS
DESTAIN O.N.



To Page No. _____

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Date _____

Invented by _____

Date _____

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Exhibit A (continued)

Project No. _____
Book No. **31884**

43

TITLE _____

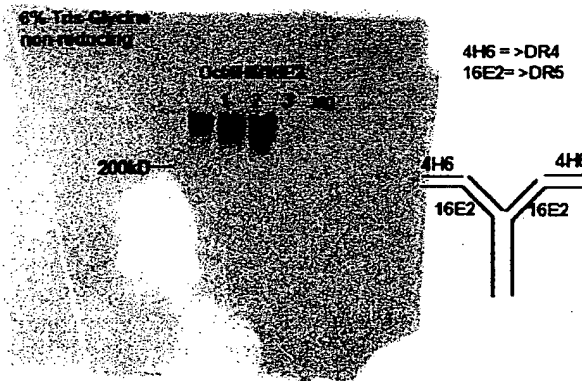
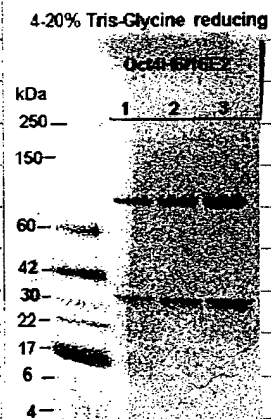
From Page No. 40

CONCENTRATE & PURIFY 4H6/16E2 OCT PROTEIN FROM 293 TRANSFECTANTS; YM100 FILTER
SEE PG. 38 FOR PROTOCOL
PURIFY OVER PROTEIN A SEPH.

$$\frac{OD\ 280}{0.139} \times 1:10 \rightarrow 800ml\ volume$$

$$\frac{(0.39)(10)}{1.38\ (EXT\ COEFF)} = 2.0\ mg/ml$$

[REDACTED]



To Page No. _____

Witnessed & Understood by me, _____

Date _____

Invented by _____

Date _____

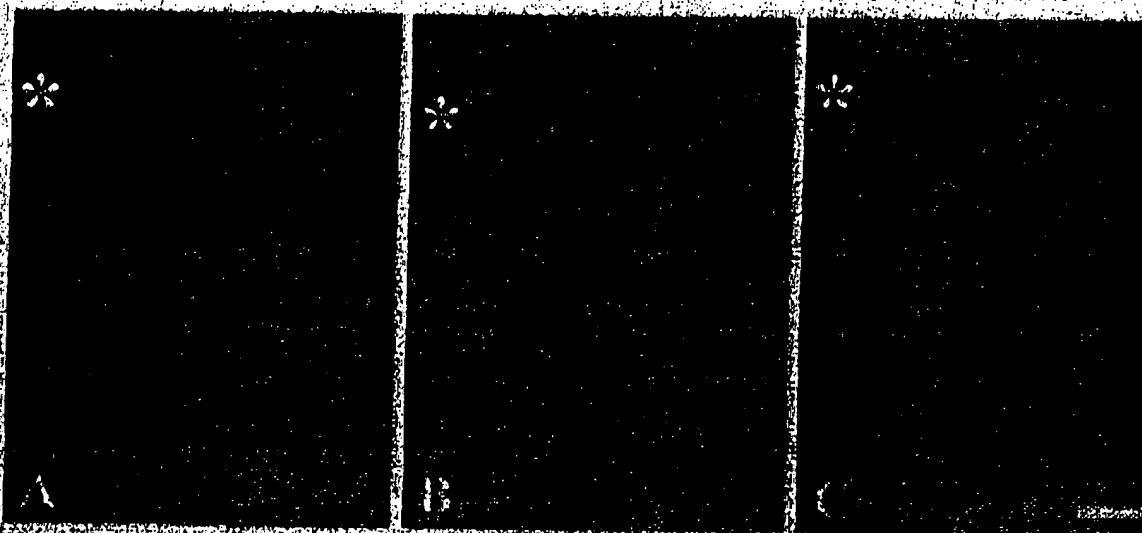
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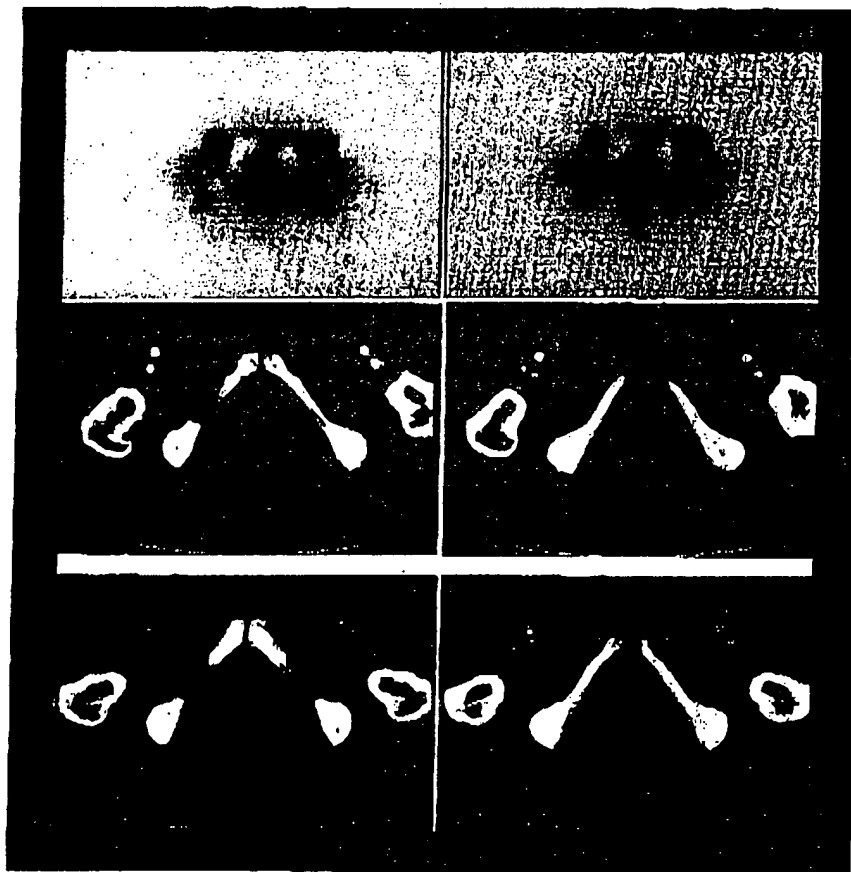
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